Identification of Aberrantly Regulated Genes in Diseased Skin Using the cDNA Differential Display Technique

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It is hypothesized that psoriasis may be caused by aberrant gene expression. In an effort to identify and clone psoriasis-specific genes, we compared gene expression in normal, tape-stripped (wounded), and psoriatic skin using the cDNA differential display technique. Four genes not previously described in psoriasis—connexin 26, a gap junction protein; squamous cell carcinoma antigen-1 (SCCA1), a serine protease inhibitor; and mitochondrial NAD subunits 5 and 6—were identified as having very high expression levels in psoriatic skin. *In situ* hybridizations showed that connexin 26 mRNA was expressed 10-fold higher in psoriatic and 4-fold higher in tape-stripped epidermis than in controls. SCCA1 showed a 40-fold increase in mRNA expression, whereas mitochondrial NAD5 and NAD6 expression was increased 10- and 20-fold, respectively, in psoriatic skin. Northern blots confirmed the increased expression of connexin 26, SCCA1, and NAD6 genes in psoriatic skin. Immunohistochemistry showed that connexin 26 protein was strongly expressed in spinous keratinocytes from psoriatic skin and chronic wounds, but was absent in normal epidermis. These studies demonstrate the usefulness of this approach for identifying genes that are conditionally expressed in growth-activated human skin. *Key words: Connexin-26/squamous cell carcinoma antigen/gap junctions/serine protease inhibitor/mitochondrial NADH5 and 6. J Invest Dermatol 108:188–194, 1997*

Psoriasis represents a complex pathologic process in which the activation of at least two sets of cells is profoundly altered: (i) keratinocytes that undergo hyperplasia and differentiation along the "alternate" regenerative maturational pathway (Mansbridge and Knapp, 1987; McKay and Leigh, 1995) and (ii) infiltrating immune cells that activate a cytokine network as part of the immune response and induce or sustain epidermal changes (Cooper, 1990; Gottlieb *et al*, 1995). To date, the search for molecular alterations in the psoriatic lesion has been based on known molecules, such as growth factors and cytokines predicted to be in these cell types (Nickoloff, 1991; Krueger and Gottlieb, 1994). One exception to this approach was a blind study in which two-dimensional gel electrophoresis was used to identify proteins with aberrant regulation in psoriasis (Celis *et al*, 1990). This approach yielded proteins with useful information, such as psoriasin (Hoffmann *et al*, 1994); however, it has technical drawbacks in that one has to perform complex reverse genetics, in which peptides of two-dimensional gel identified proteins are sequenced to design a putative DNA probe to obtain the cDNA of the protein. We used the mRNA differential display technique (Liang and Pardee, 1992) and the secondary screening methods of *in situ* hybridization and northern blots to clone and characterize genes involved in the psoriatic phenotype without bias to prior identity. With the latter approach, one clones cDNAs of psoriasis-specific genes directly without having to sequence protein products.

Because psoriasis has many features in common with wound healing (Mansbridge and Knapp, 1987; Nickoloff and Naidu, 1994; McKay and Leigh, 1995), we have included wounded skin, generated by tape-stripping, in our cDNA screening. In the normal wound-healing process, the regenerative pathway of keratinocyte maturation is transient and the epidermis returns to its normal growth and differentiation program (Mansbridge and Knapp, 1987). In psoriatic skin, the regenerative pathway is persistently activated (Mansbridge and Knapp, 1987; McKay and Leigh, 1995). To determine whether identified genes are disease-specific in psoriasis, it is necessary to compare these genes with those expressed during "physiologic" hyperplasia. Those found in both may be part of the regenerative pathway; those found only in psoriatic skin are potential candidates for the molecular regulators of the diseased state.

In this report we present the isolation and characterization of four differentially regulated genes in psoriatic and wounded skin whose expression in psoriasis was previously unknown. These are connexin 26, squamous cell carcinoma antigen 1, and mitochondrial nicotine adenine dehydrogenase subunits 5 and 6.

MATERIALS AND METHODS

Human Skin Samples Skin biopsies (6-mm punches) from five psoriatic patients and five normal volunteers were obtained for this study with the

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Abbreviations: SCCA1, squamous cell carcinoma antigen 1.

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patients' informed consent and IRB approval at facilities of The Rockefeller University Hospital. Patients and volunteers (eight males and two females) were between the ages of 21 and 50 and of various ethnic backgrounds; biopsies were taken from the leg, except one, which came from the arm of a volunteer; psoriatic biopsies were from pre-treatment patients with chronic plaque-type psoriasis.

To create a superficial wound, we removed the stratum corneum of normal volunteers and unaffected skin of psoriatic patients by tape-stripping 50–150 times until a glistening interface appeared, according to a standard protocol (Nickoloff and Naidu, 1994). Twenty-four hours later, two neighboring 6-mm punch biopsies were obtained from wounded and unwounded areas, as well as from psoriatic skin of patients. Of the two biopsies, one was processed for differential display, and the other was quickly frozen in tissue tek OCT (Miles, Elkhart, IN). The induction of the regenerative maturational pathway in tape-stripped skin was verified by the presence of high levels of keratin 16 (Gottlieb *et al*, 1996) using immunocytochemistry (data not shown) and by absence of the stratum corneum. For differential display there were a total of five groups where n = 3 per group: Group 1, normal skin; Group 2, normal skin tape-stripped; Group 3, unaffected skin of psoriatic patients; Group 4, unaffected skin of psoriatic patients tape-stripped; Group 5, psoriatic skin.

For *in-situ* hybridizations, an additional one to two samples (n = 4–5 total) were added to each group from other patients and volunteers to strengthen statistical analysis.

mRNA Differential Display The polymerase chain reaction (PCR)-based mRNA differential display technique was performed by the method of Liang and Pardee (1992) with our modifications (Mello *et al*, 1997). To minimize RNA degradation (Elder *et al*, 1990), skin biopsies were immediately homogenized with a Kinematica homogenizer (Brinkman, Westbury, NY) after removal from the patient, and total RNA was isolated by the method of Chomczynski and Saachi (1987). Contaminating DNA was removed by treating the RNA with deoxyribonuclease I, using the message clean kit of GenHunter (Brookline, MA). cDNAs were then synthesized in a 40-μl RNAase-free reaction containing 100 ng of deoxyribonuclease-treated total RNA, 250 μM deoxynucleoside triphosphates (Pharmacia; Piscataway, NJ), 10 μM dithiothreitol (Sigma; St. Louis, MO), 1 U of RNAsin/μl (Promega; Madison, WI), 1 μM of one of four oligo $dT_{12}MN$ degenerate primers (where M = G, A, or C, and N = G, A, T, or C), 1× Superscript Buffer, and 10 U of Superscript II reverse transcriptase (GIBCO BRL; Gaithersburg, MD). The reactions were incubated for 30 min at 42°C. The cDNAs were amplified by PCR in a 10-μl reaction containing the same $dT_{12}MN$ primer (1 μM), 0.2 μM of one of 20 arbitrary 10 mer primers of random sequence (AP1-20; GenHunter), 1 μl of the cDNA reaction, 5 μCi of [^{35}S]dATP (NEN-Dupont; Boston, MA) 1× *Taq* polymerase buffer I, an additional 0.5 mM $MgCl_2$, and 0.1 U of *Taq* polymerase (Perkin-Elmer; Branchburg, NJ). The reaction was cycled at 95°C for 30 s; 40°C for 2 min; 72°C for 30 s; for 40 cycles. It was then extended for 7 min at 72°C and then held at 4°C in a Perkin-Elmer 480 thermocycler. Before gel loading, 5 μl of sequencing loading dye were added, the sample was denatured by heating to 80°C for 2 min, and 7.5 μl were electrophoresed for 2.5–3 h on a 6% polyacrylamide 7 M urea-wedged sequencing gel. Gels were dried without fixation and exposed to x-ray film for 1–4 d. The sequences of the AP and $T_{12}MN$ primers used were obtained from the Differential Display kit provided by GenHunter. Primers were synthesized at the Protein and DNA sequencing facilities of the Rockefeller University.

Our criterion for differentially regulated PCR fragments was to select those bands present in at least two of the three psoriatic and/or wound-healing samples and absent from any control samples (or vice-versa). We found this was the best way to guard against isolating false positives that are due to natural genetic variability among people and/or endogenous PCR variability. These fragments were excised from the gel, heated in 100 μl of water for 10 min, cooled to room temperature, and briefly spun. The supernatant was transferred to a fresh microfuge tube and the fragment precipitated in the presence of 10 mM glycogen, 0.3 M Na acetate, and 2 vol of ethanol (EtOH) at −20°C for 30 min. The DNA was pelleted, washed with 70% EtOH, dried, and resuspended in 10 μl of sterile dH_2O. The sizes of the picked bands were estimated relative to the xylene cyanol dye on a 6% wedged sequencing gel, which co-migrates with 123 DNA bases. The excised bands were re-amplified in a 40-μl reaction with the same set of primers used to generate it. Reactions containing the correct size band were cloned into the pCRII vector as recommended by the supplier (Invitrogen; San Diego, CA). Separately, 30 μl of the re-amplified band were electrophoresed in a 2% agarose gel and purified with the Qiagen kit (Qiagen, Chatsworth, CA) for use as a probe to verify pCRII recombinants. The bands were given SK (Skin) numbers according to the order they were picked.

***In Situ* Hybridizations** As a secondary screening approach, *in situ* hybridizations were performed with riboprobes according to the method of Mello and Clayton (1995) with modifications by Jarvis *et al* (1995). Secondary screening via *in situ* analysis is the preferred method, because one biopsy can generate many sections (300) for screening many genes. Screening by northern blotting is not advantageous, due to inherent limits in material. Riboprobes of cloned fragments were generated from both strands by restriction of pCRII recombinants with *Bam*HI or *Xba*I (New England Biolabs; Boston, MA) and transcribed in the presence of 50 μCi of [^{35}S]UTP (NEN-Dupont; Boston, MA) in 1× transcription buffer [0.5 mM ATP, CTP, and GTP and 60 μM UTP (Pharmacia), 10 mM dithiothreitol (Sigma; St. Louis, MO), 40 mM Tris(hydroxymethyl)aminomethane-HCl, pH 7.5, 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, and 0.05 mg BSA per ml], 1 μg of restricted DNA, 50 U of T7 RNA polymerase or 80 U of SP6 RNA polymerase (Promega; Madison, WI), respectively, and incubated at 42°C for 1 h. When using the pCRII vector of Invitrogen, we found it necessary to use the high concentration SP6 RNA polymerase of Promega, as pCRII has a weak SP6 promoter. Probes were purified through G-50 spun columns.

Glass microscope slides were prepared for sectioning by being washed in 1 N HCl for 20 min, rinsed in dH_2O, dehydrated in 100% ethanol for 20 min, air-dried, coated with 2% TESPA (3-aminopropyl-triethoxysilane; Aldrich Chemical Co., Milwaukee, WI), rinsed 2x in acetone and 1x in water, and air-dried. Ten-micron frozen sections were cut on a cryostat and mounted onto TESPA coated slides. Sections were stored frozen at −70°C. On the day of hybridization, all sections to be hybridized with any given probe were fixed at the same time in a 3% paraformaldehye phosphate-buffered saline solution for 5 min. The slides were washed twice in phosphate-buffered saline and then acetylated in 100 mM triethynolamine, 25 mM acetic anhydride solution within 10 s of mixing reagents. Slides were washed three times in 2× SSPE, then dehydrated in 70, 95, and 100% ethanol. To each section was added hybridization solution, containing 50% formamide, 2×SSPE [1× = 0.18 M NaCl, 10 mM NaPO4 (pH 7.5), 1 mM Na EDTA], 2 μg per ml tRNA, 1 μg per ml BSA, 0.4 μg per ml poly A, 100 mM dithiothreitol, 0.5 × 10^6 cpm of riboprobe in a 16-μl volume per section. Sections were then coverslipped and incubated under mineral oil at 50°C for 3 h. Oil was removed in two rinses of chloroform and slides decoverslipped in 2× SSPE containing 0.1% β-mercaptoethanol. Slides were then washed for 1 h at room temperature in 2×SSPE and 0.1% β-mercaptoethanol, washed 1 h at 50°C in 50% formamide, 2×SSPE, and 0.1% β-mercaptoethanol, followed by two 30-min washes at 50°C in 0.1×SSPE. Slides were then exposed for 3 d on a PhosphoImager screen and scanned (Molecular Dynamics, Sunnyvale, CA). Sections that showed a positive result with one of the riboprobes (presumed antisense) consistent with the differential display reaction were placed twice in xylene for 5 min, hydrated by decreasing ethanol concentrations for 2 min each (100%, 95%, 70%, 50%, H_2O), air-dried, dipped into photographic emulsion (Kodak NTB2, Eastman-Kodak, Rochester, NY), and exposed for 3–6 wk. After photographic development, sections were counter-stained with 0.13% cresyl violet 3–5 min, rinsed in water, and dipped a few times in increasing ethanol concentrations (50%, 70%, 95%, twice in 100%), in xylene twice for 5 min, and coverslipped with Permount (Fisher Scientific; Fair Lawn, NJ).

Quantification Emulsion-dipped slides were analyzed using the image analysis software, NIH Image 1.5, written by Wayne Rasband. Grains per cell were counted from four adjacent cells in each epidermal layer (basal, spinous, granular, and transitional layer of psoriatic plaques) and cells in the dermis close to the basement membrane for each skin section, under oil immersion with a 63× objective. Active psoriatic epidermis lacks a granular layer, but there is a clear spinous-to-cornified boundary that is termed "transitional layer" in this report. Individual patient values are averages from measures made in four contiguous cells, while group means are derived from average values of all patients. An unpaired Student's t test was used to test for significant differences between experimental groups using the Statview program from Brainpower Inc. (Calabasas, CA). A paired t test was used to test for differences between skin layers of the same group.

DNA Sequencing PCR fragments cloned into the pCRII vector were sequenced via the CircumVent sequencing kit (New England Biolabs; Boston, MA) with SP6 (Invitrogen; San Diego, CA), and T7 (New England Biolabs) promoter primers. The sequences were compared to those stored in DNA sequence databases via the BLASTN and FASTA programs of the NIH server (Altschul *et al*, 1990).

Northern Analysis Ten micrograms of total RNA from psoriatic and unaffected skin were electrophoresed on a 1% agarose MOPS formaldehyde gel (Thomas, 1980). The gel was soaked in 20× SSPE for 1 h and blotted onto a Genescreen Plus nylon membrane (NEN-Dupont, Boston, MA) and hybridized with a riboprobe using a previously described method (Clayton

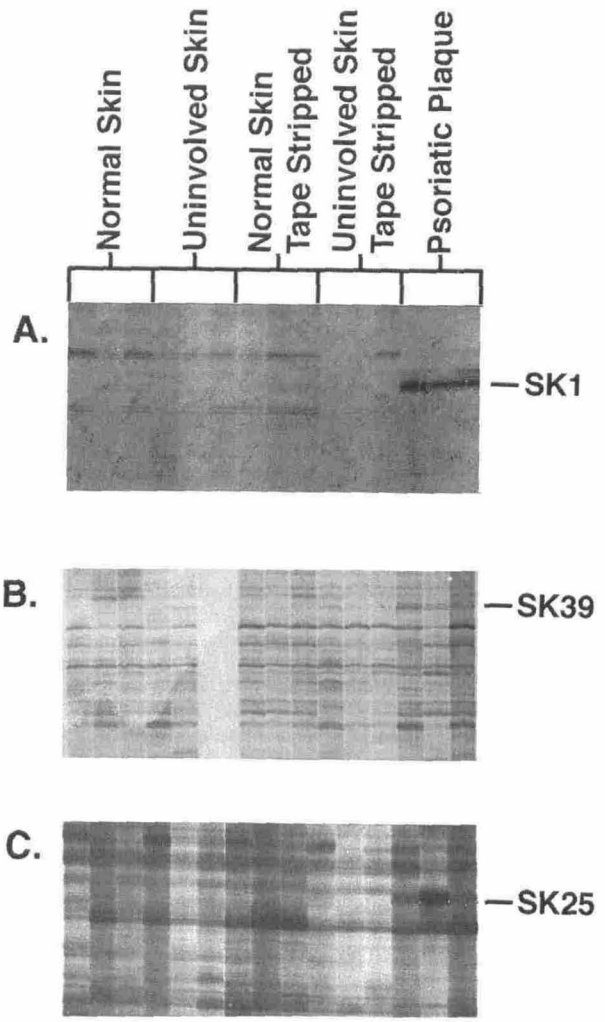

Figure 1. Differential display gels. Autoradiograms of mRNA differential display gels showing relevant areas where differentially expressed bands were excised for this study. RNA from normal skin, normal tape-stripped skin, unaffected psoriatic skin, unaffected psoriatic skin tape stripped, and psoriatic skin was converted to cDNA with SuperscriptII Reverse Transcriptase and oligo $dT_{12}MC$ and PCR amplified with arbitrary primer AP4, 5′-GGTACTCCAC-3′, AP6, 5′-GCAATCGATG-3′ and AP5, 5′-GTTGCGATCC-3′, respectively; see *Materials and Methods* for details. *(A)* SK1, *(B)* SK39, and *(C)* SK25 were picked as bands that showed increased expression in three of three psoriatic patients.

et al, 1988). Riboprobes were generated in the same reaction conditions described for *in situ* hybridizations, except that the labeled nucleotide was [^{32}P]UTP instead of [^{35}S]UTP. Hybridization and washing temperatures were 55°C.

Immunocytochemistry Immunocytochemistry was performed essentially as previously described (Gottlieb *et al*, 1995). Rabbit antibodies to rat connexin 26 (1.5 mg per ml) and connexin 43 (1.8 mg per ml) were a kind gift of Dr. Gilula (Risek *et al*, 1990; Nishi *et al*, 1991) and were diluted at 1:500 and 1:1000, respectively. Antibodies to rat connexins 32 and 40 were kind gifts of Dr. Paul and used as described (Golinger and Paul, 1995).

RESULTS

A screen using a subset of available primers (Liang and Pardee, 1992) yielded >40 cDNA products that appeared to differ between normal skin and psoriasis or tape-stripped skin. **Figure 1** illustrates the appearance of some differentially expressed cDNA products as visualized in autoradiograms of gels. Of these products, 20 were selected for further analysis, based on apparently exclusive expression in one or more related groups. From this group, eight were confirmed to be differentially regulated based on *in situ* hybridization. In this report we present the selective expression of four of these gene products whose sequence corresponds to previously identified genes in other biologic systems but whose expression in psoriatic epidermis has not been previously described. The enhanced expression of two of these genes, connexin 26 and squamous cell carcinoma antigen 1 (SCCA1), would not have been predicted based on known cellular alterations in psoriasis, whereas expression of the other two genes, mitochondrial nicotine adenine dehydrogenase subunits 5 and 6 (mtNAD5 and mtNAD6), might have been anticipated based on increased metabolic requirements of growth-activated epithelium. The other four clones are novel gene products and will be reported on separately after further characterization.

SK1 (Connexin 26) The PCR fragment designated SK1 was selected due to its robust appearance in three of three lesional psoriatic samples and its minimal-to-absent expression in cDNA reactions from other skin sources **(Fig 1*A*)**. *In situ* hybridizations with SK1 riboprobes **(Figs 2*A*, 3*A*)** showed minimal expression in controls, a small increase in tape-stripped skin, and high expression in the epidermis of psoriasis. This increase occurred in a gradient fashion from basal to spinous layers in both tape-stripped and psoriatic skin. The levels in the spinous layer of psoriatic skin were 5-fold higher than in tape-stripped skin ($p < 0.01$) and 10-fold above controls ($p < 0.001$). Expression in tape-stripped epidermis was only 2-fold higher than in controls ($p < 0.02$). The sequence of the SK1 cDNA (164bp) sense strand was identical to the 26-kDa gap junction protein, *connexin 26* (from nucleotides 2147 to 2310) (Lee *et al*, 1992). The SK1 riboprobe detected a single 2.4-kb mRNA in northern blots from lesional psoriasis consistent with the size of the *connexin 26* mRNA (Lee *et al*, 1992), whereas no mRNA was detected in unaffected skin **(Fig 4)**. Thus these results demonstrate that SK1 is *connexin 26*.

To determine whether or not a connexin 26 protein product is synthesized in different conditions, we performed immunohistochemistry with an antibody to connexin 26 on several skin substrates. Increased expression of connexin 26 protein was observed consistently in eight of eight psoriatic skin samples **(Fig 5)**, which paralleled the mRNA expression pattern of *in situ* hybridization (cf. **Figs 2*A*** and **3*A***). The connexin 26 protein appeared in membrane regions between keratinocytes, as would be expected for gap junctions. Connexin 26 protein was not observed in epidermal keratinocytes of normal or unaffected skin, but faint staining of appendageal keratinocytes, e.g., acrotrichial keratinocytes associated with hair follicles, was observed **(Fig 5)**, in accord with prior reports (Salomon *et al*, 1994). Surprisingly, connexin 26 protein expression was not detected in epidermal keratinocytes of tape-stripped skin (not shown), although relatively low mRNA levels were found by *in situ* hybridizations. In hyperplastic epidermis from chronic wounds, however, connexin 26 protein was detected in spinous keratinocytes, but still at lower levels than in psoriatic skin **(Fig 5)**.

For comparison, we assayed the expression of connexins 43, 40, and 32 in adjacent skin sections from the same sources **(Fig 5)**. Connexin 43 was constitutively expressed by spinous keratinocytes of all tissue samples examined, but its expression in psoriatic epidermis may have been increased somewhat relative to skin controls. Proteins for connexins 32 and 40 were not expressed in either normal or psoriatic epidermal keratinocytes; but like connexin 26, connexin 32 was present in the appendageal keratinocytes of all skin groups (data not shown). Hence, keratinocyte connexin 26 expression is induced in epidermis of psoriatic skin, but its expression does not replace the normal expression of connexin 43.

SK39 (Squamous Cell Carcinoma Antigen-1) SK39 is a cDNA fragment that appeared in psoriatic plaque reactions **(Fig 1*B*)**. Quantitative analysis **(Fig 3*B*)** of *in situ* hybridizations **(Fig 2*A*)** showed that SK39 increased 40-fold in psoriatic skin. The highest expression was in the upper spinous layers and the transitional zones between the spinous and cornified layers ($p < 0.002$;

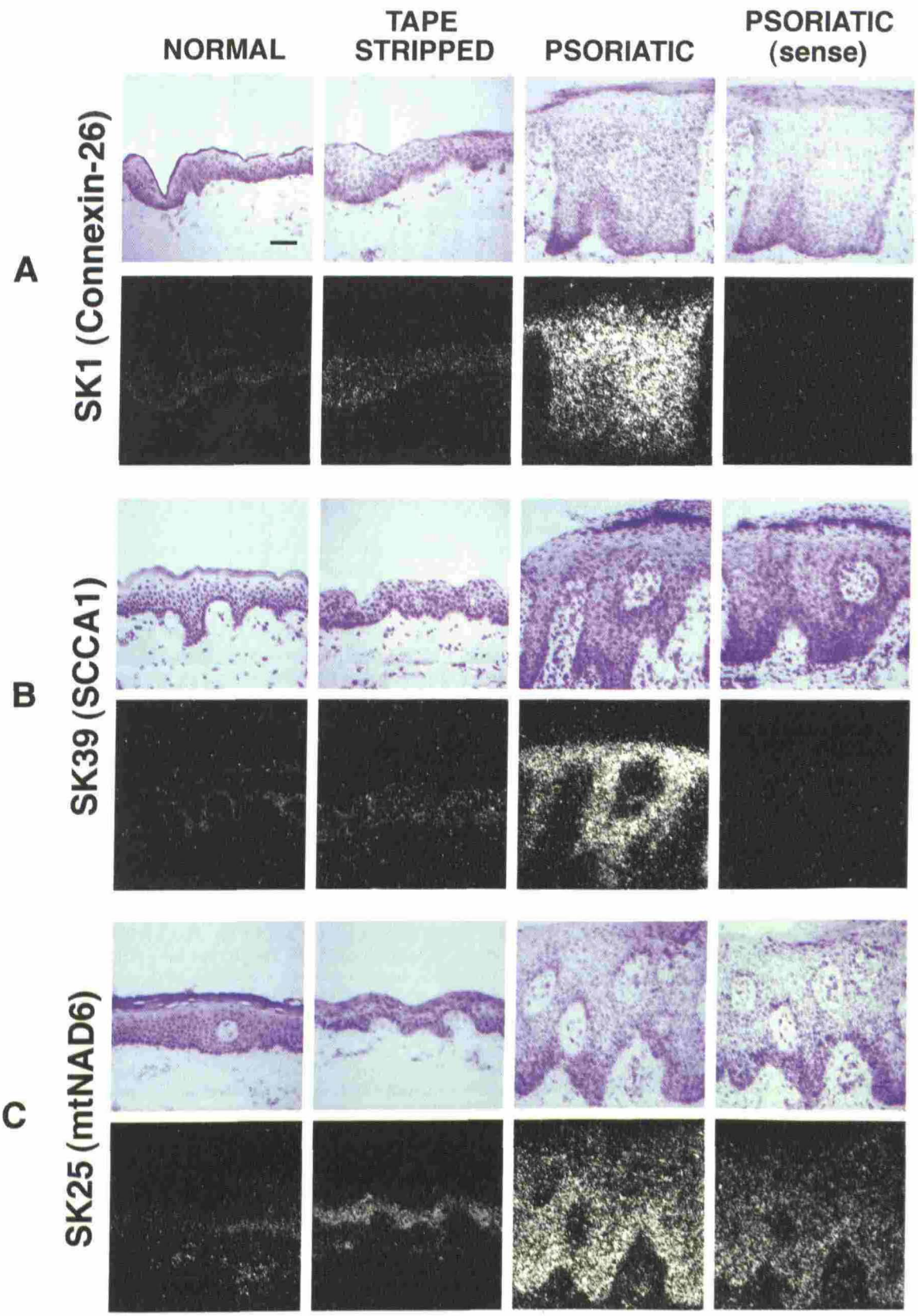

Figure 2. *In situ* hybridizations of differentially regulated clones. There is increased *(A)* SK1 (connexin 26), *(B)* SK39 (SCCA1), and *(C)* SK25 (mtNAD6) mRNA expression in wounded and psoriatic skin and mtNAD5 in psoriatic skin (sense strand of mtNAD6). Emulsion-dipped slides were hybridized with ^{35}S-labeled antisense and sense riboprobes. The first and second row of each panel show bright- and darkfield photos, respectively, of cresyl violet-stained skin sections. Note the dramatic increase in grain intensity in psoriatic skin for each mRNA (darkfield section). Note also the removal of the upper stratum corneum layer in tape-stripped skin. *Scale bar*, 100 μm.

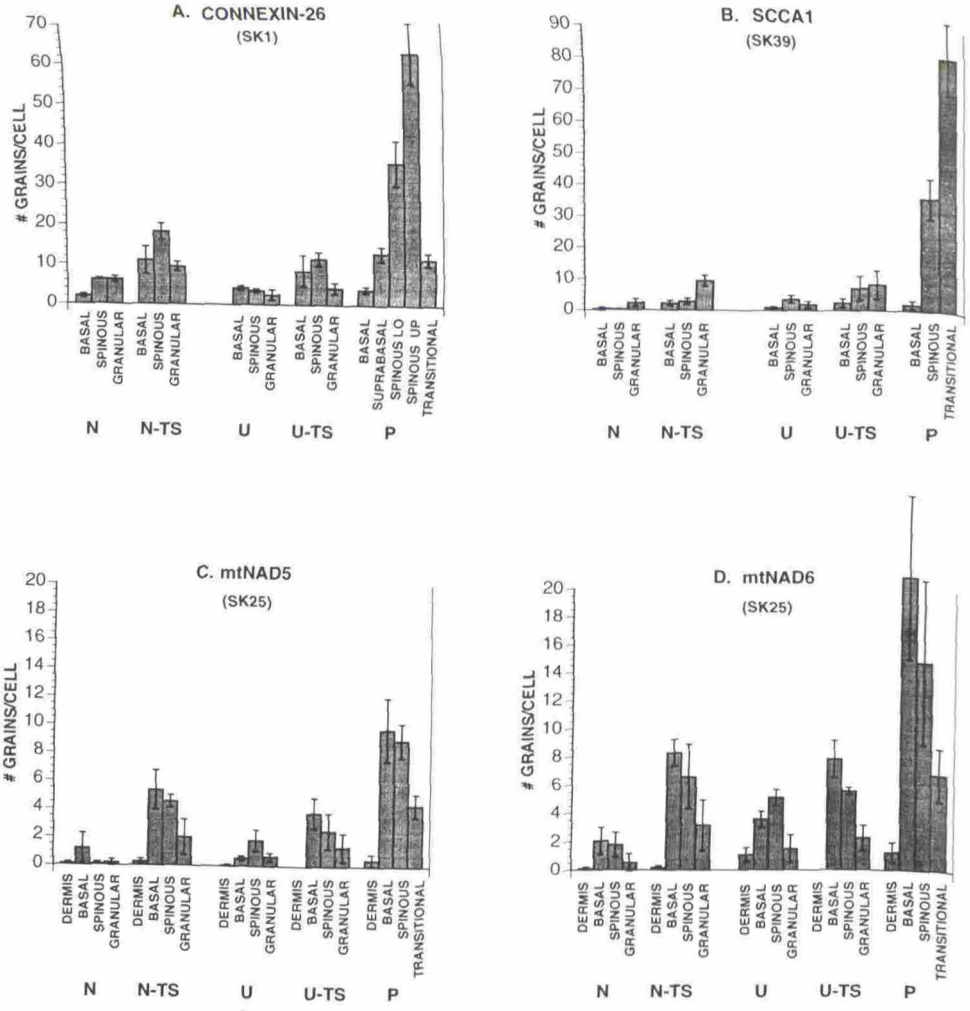

Figure 3. Quantification of *in situ* hybridizations. Gene expression levels of *(A)* SK1 (connexin 26), *(B)* SK39 (SCCA1), *(C,D)* SK25 (mtNAD5 and mtNAD6). The relative increase in tape-stripped and psoriatic skin differed for different genes and differed in particular skin layers. The transitional zone defines the area from the spinous to parakeratotic layer of psoriatic skin. Bars represent the averages of the total (#) grains per cell. Error bars represent SEM. N, Normal skin (n = 5); N-TS, normal tape-stripped skin (n = 4); U, unaffected skin of a psoriatic patient (n = 5); U-TS, unaffected tape-stripped skin of a psoriatic patient (n = 3); p, psoriatic skin (n = 5).

Figs 2*B*, 3*B*). Much smaller, but significant, elevations were observed in the granular layers between controls and tape-stripped specimens ($p < 0.01$). Sequence analysis showed that SK39 (274 bp) was identical to squamous cell carcinoma antigen-1 (SCCA-1) from nucleotides 909 to 1182. SSCA-1 has been identified as a serine protease inhibitor (Suminami *et al*, 1991; Schneider *et al*, 1995). The SK39 probe hybridized to a single 1.5-kb mRNA in psoriatic skin, consistent with the size of SCCA1 mRNA, but no hybridization was seen in mRNA from unaffected skin of psoriatics **(Fig 4)**.

SK25 (Mitochondrial NAD Subunits 5 and 6) SK25 was also chosen based on exclusive cDNA amplification from psoriatic plaque **(Fig 2*C*)**. In contrast to the two above clones, *in situ* hybridization with SK25 showed increased expression in psoriasis, with both strands with the highest expression in the basal cell layer and decreasing expression in the more differentiated layers **(Fig 2*C*)**: 10-fold with one strand ($p < 0.001$) and 20-fold with the other ($p < 0.001$). The nucleotide sequence of SK25 (208 bp) was virtually identical to mitochondrial NAD subunits 5 and 6 (from nucleotides 13,208 to 13,415) (Anderson *et al*, 1981; Chomyn *et al*, 1985; Chomyn *et al*, 1986). Due to tightly conserved coding in mitochondrial DNA, the strand opposite NAD5 encodes part of NAD6. Hence the "sense" riboprobe strand of NAD5 is the antisense probe of NAD6, which explains why both strands had hybridized. The mitochondrial NAD5 and NAD6 are expressed at their highest levels in the proliferating basal cell layer, in contrast to connexin 26 and SSCA 1, which were more highly expressed in the more differentiated spinous and transitional layers, respectively. Similar to the clones above, *in-situs* with NAD5 and 6 showed a small increase in tape-stripped skin, although expression was not detected in the differential display gels. Northern analysis with the NAD6 strand showed hybridization to two bands in psoriatic plaque of sizes 1.2 and 5.0 kb. The 1.2-kb size is consistent with the NAD6 mRNA; the 5.0 kb may be a larger unprocessed transcript containing upstream sequences of the neighboring co-transcribed gene, tRNA-glu (Anderson *et al*, 1981).

DISCUSSION

The power of the cDNA differential display technique to directly identify and isolate differentially expressed genes involved in diseased psoriatic skin is clearly demonstrated here. This methodology is subject to a number of potential artifacts that can lead to false positives (Liang and Pardee, 1995). To guard against these, we performed reactions in triplicate and found that cDNA bands with strong expression in three of three samples from a group reflected legitimate differences in gene expression that were confirmed by *in situ* hybridization and northern analysis. Although northern analysis was required to confirm mRNA sizes of known gene products, it consumed a relatively large amount of tissue (all of a 6-mm punch biopsy) for total RNA preparation and was not suitable as a secondary screening technique. In contrast, *in situ* hybridization was more advantageous for secondary screening, since the same 6-mm punch biopsy could be used to confirm differential expression of approximately 150 cDNA gene products (i.e., 300 sections) and could give both quantitative information and anatomical localization of cells that express particular mRNAs. For tape-stripped skin,

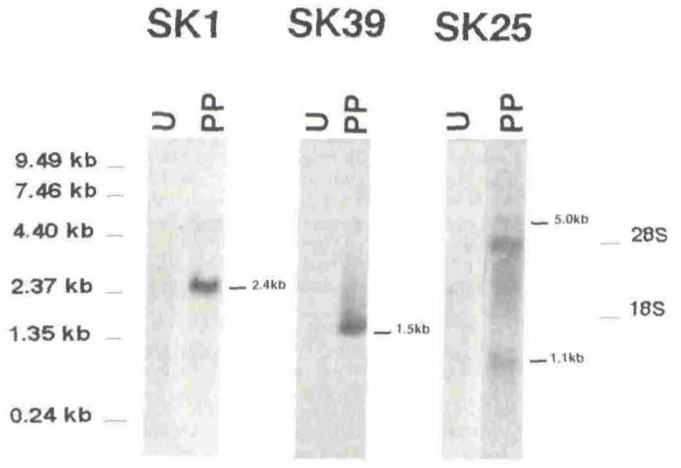

Figure 4. Northern hybridizations of total RNA from unaffected and psoriatic skin. Line marks indicate the migration of 28S and 18S ribosomal RNA and of the RNA ladder size marker. Increased expression of SK1 (*connexin 26*), SK39 (SCCA1), and SK25 (mtNADH6) in the psoriatic plaque confirmed the *in situ* hybridization patterns, and the sizes of the mRNAs confirmed the identity of each cDNA clone as determined by sequence and Genbank analysis.

differences in gene expression detected by *in situ* hybridization appeared to be more sensitive than detection by differential display or protein detection by immunocytochemisty. On the other hand, without differential display these genes would not have been revealed. Finally, the size of the cDNA products obtained (>150 nucleotides) was usually sufficient to sequence and to compare identity with published sequences of known gene products. Small fragments of unknown gene products are not sufficient to report, but are sufficient as a probe to obtain the full-length clones from a psoriatic skin library. Products that were identified in psoriatic tissue in this study, connexin 26, SCCA1, mtNAD5, and mtNAD6, had not been previously detected in psoriasis and would not necessarily have been predicted based on known properties of cellular alterations in this disease. The potential biologic relevance of these gene products in psoriasis is discussed below.

Gap junctions are communication membrane channels that transmit intercellular signals between two adjacent cells. They are formed through self-association of one or more of 12 connexin proteins and are found in many cell types: from opposing epithelial cells to synaptic connections between neurons (for review see Kumar and Gilula, 1996). Electron micrographs of freeze-fractured cells from unaffected and psoriatic skin identified larger and more numerous gap junctions in the cell membranes of psoriatic keratinocytes compared to unaffected keratinocytes (Caputo *et al*, 1978), but the molecular composition of these psoriatic gap junctions had not been determined. The results of this study suggest that the gap junctions of psoriatic epidermis found in their study are composed of connexin 26 and 43, unlike those in normal interfollicular epidermis, which consist only of connexin 43 protein (Salomon *et al*, 1994).

Recently, it has been shown that wound healing in rat tail skin is accompanied by increased expression of connexin 26, decreased expression of connexin 43 at wound edges, and increased permeability of gap junctions (Golinger and Paul, 1995). The expression of connexins 26 and 43, however, differs significantly in human skin and rodent skin. In the latter, connexin 26 is expressed in granular keratinocytes, while connexin 43 is expressed by basal and lower spinous keratinocytes (Risek *et al*, 1994). We speculate that the expression of connexin 26 in human epidermis could increase intercellular permeability via gap junctions and might, thus, contribute directly to keratinocyte activation in psoriatic or regenerative epidermis.

The squamous cell carcinoma antigen-1 (SCCA-1) was initially identified in keratinocytes of squamous cell carcinoma (SCC) from the cervix, and its expression is used to diagnose or monitor clinical growth of SCCs of patients from different tissues (Kato and Torigoe, 1977). The SCCA-1 protein is a serine protease inhibitor whose expression might confer some protective effects to epithelial cells. Interestingly, its expression appears to be inducible by cytokines or other soluble factors released from peripheral blood leukocytes (Suminami *et al*, 1991), a finding that might explain its

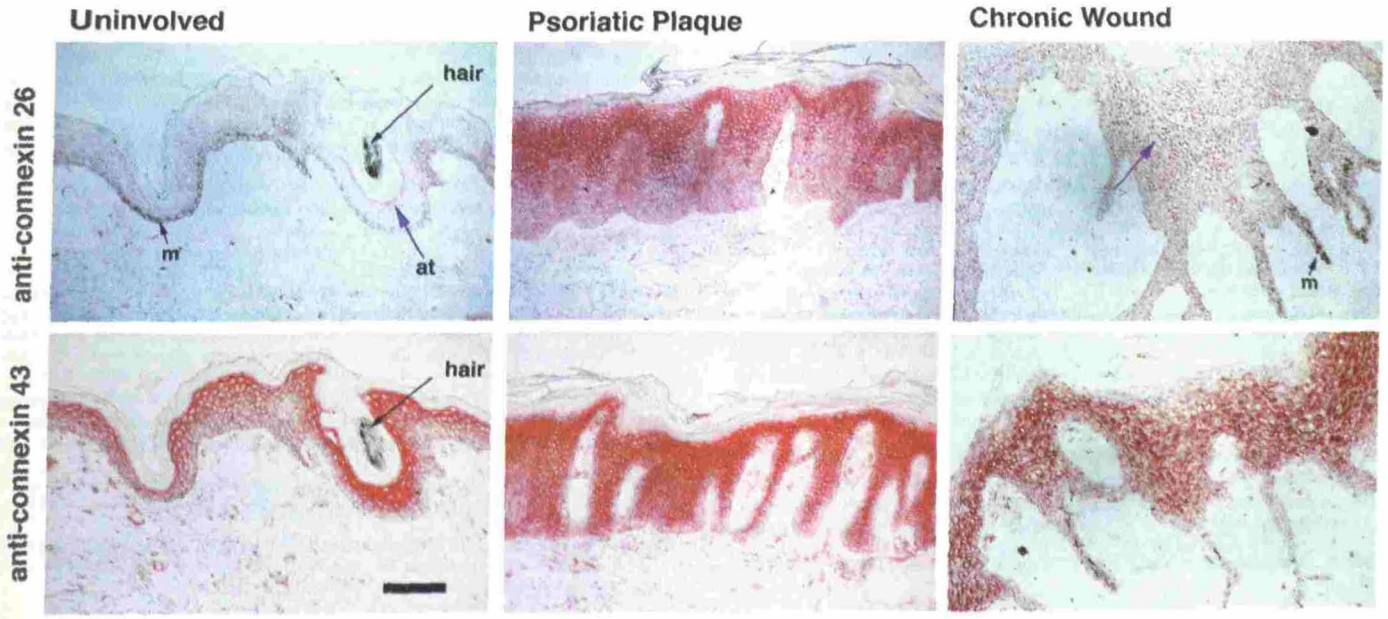

Figure 5. Immunohistochemistry showing connexin 26 and connexin 43 protein expression. Skin sections from unaffected skin, chronic wound, and psoriatic plaque were immunohistochemically stained with antibodies to connexins 26 and 43. Increased connexin 26 protein was found at the cell membranes of psoriatic and chronic wounded skin. A small increase was also detected for connexin 43. *Scale bar*, 100 μm; at, acrotrichial epithelium; m, melanin.

high expression in psoriatic lesional epidermis (due to leukocytic infiltration of psoriatic skin). Furthermore, it is known that neutrophils and some T cells have granules containing serine proteases (Henkart *et al*, 1987). Given its function as a serine protease inhibitor, high-level expression of SCCA-1 in epidermal keratinocytes might provide some protection from serine proteases released from activated leukocytes at sites of cutaneous inflammation. This could be particularly important to prevent damage by activated neutrophils, which accumulate in psoriatic epidermis immediately adjacent to epidermal regions expressing high SCCA-1 mRNA. One could also speculate that high levels of a serine protease inhibitor in psoriatic tissue might limit conversion of some pro-cytokines, e.g., interleukin-1 or transforming growth factor-β, to active forms and thus indirectly influence the inflammatory or pro-growth environment in psoriatic lesions.

Increased expression of mitochondrial NAD5 and NAD6 could be due to an increase in the number of mitochondria per cell, to an increase in the number of gene transcripts per mitochondria, or to a combination of these factors. The first scenrio seems plausible, considering that there are more mitochondria per cell in keratinocytes of psoriatic plaques (Morliere *et al*, 1985).

This report illustrates that the mRNA differential display technique is a useful method to isolate genes directly involved in the diseased hyper-proliferative state of psoriasis and the normal hyper-proliferative state of wound healing. By comparing gene expression in psoriatic and tape-stripped skin with gene expression in normal skin, one can begin to obtain a collection of genes, known and unknown, that are conditionally or selectively modulated. In turn, one can begin to ask questions about their functions in normal, regenerative, or diseased skin.

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